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ON THE PROBLEM OF SEXUAL DIFFERENTIATION

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ON THE PROBLEM OF SEXUAL DIFFERENTIATION*

R. Hertwig

ABSTRACT. The problems of sexual differentiation of daphnids, Dinophilus apatris, frogs, Rana temporaria, and Rana esculenta are experimentally investigated. The important points from two earlier works on protozoa are summarized by way of introduction. The various aspects of cell function affecting sexual differentiation, such as nuclear-plasmic relationships, cell division, assimilatory activity, etc. and environmental factors, such as feeding, temperature and egg size, are considered. It was found, for example, that warmth favors the development of male eggs of Dinophilus apatris.

The experimental investigations on the causes of sexual differentiation, about which I would like to lecture here, were stimulated by general considerations which are derived from my work with protozoa. I summarized this material in two articles which appeared two years ago under the following titles: 1) "On the Correlation of Cell and Nuclear Size," 2) "On the Exchange Relationship Between Nucleus and Protoplasm," in Biologisches Centralblatt and in Sitzungsberichten der Gesellschaft für Morphologie und Physiologie zu München /186+
I will recapitulate the most important points from these articles in order to make the experimental question understandable upon which my experiments are based. /187

I. Introduction

After I had come to the conclusion, based on culture experiments with protozoa, that there was a normal size for the nucleus of a cell, that a superabundant increase in the nuclear mass causes non-functioning of the cell, and therefore makes necessary reorganization of the cell in protozoa, of which reproduction is the most important, the extensive experiments of Gerasimoff on Spirogyra and the opportune investigations in other directions of Boveri which gave information¹ on sea urchin eggs made it possible for me to clarify my thoughts. I introduced the concept of the "nucleus plasma relation." This concept purports to say that there is a certain size relationship of nuclear mass to cell mass for every cell, which can be expressed by the quotient k/p (i.e., nuclear mass/protoplasm mass). I assumed that this quotient had the same size at corresponding phases of cell life, but that it experiences changes in a regular way corresponding to the changing functional status of the cell. Changes in the nucleus-plasma relationship depend above all on the assimilatory activity of the cell and cell distribution. I distinguished between a functional growth, i.e., one caused by metabolism of the cell, and a divisional growth of the nucleus.

*From a lecture by R. Hertwig, Munich; Transactions of the German Zoological Society, Fifteenth Annual Meeting held in Breslau, 13-16 June, 1905.

¹These have been reported meanwhile by Boveri in detail and discussed with regard to their meaning for the cell problem.

†Numbers in the margin indicate pagination in the foreign text.

All cell functions are ultimately rooted in the assimilatory activity of the cell, which causes an increase in the functioning parts of the cell, of the protoplasm and its components and formation products. Since in my experience the nuclei of very actively functioning cells increase in size, the function of the cell cannot be caused by the fact that parts of the nucleus are given up to the protoplasm, but rather the opposite, i.e., parts of the protoplasm are transferred to the nucleus. This increase in the nucleus I called its functional growth; this remains true as long as the assimilatory activity of the cell is applied to an increase in the size of the cell, i.e., as in protozoa, to the increase in protoplasm which governs the life processes. Thus a faulty relationship arises between nuclear size and cell size which I have named nuclear plasmic tension. I believe that in this fact lies the cause of cell division. When a certain degree of nuclear plasmic tension is reached, the nucleus gains the ability to grow at the cost of the protoplasm. The movements permitted by it are those which lead to division of the cell.

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A large number of remarkable phenomena can be explained by a single point of view operating from the standpoint of this newly-formulated interpretation. 1) If the division of the cell is suppressed at a critical stage by damaging influences, i.e., the nuclear plasmic tension is equalized without coming to a division, then renewed growth of the cell -- corresponding to double the size of the nucleus reaching double the normal division size of the cell -- is required to produce that degree of nucleus tension which is needed for the division of the cell. 2) If the assimilatory activity of the cell serves only partly or not at all for growth of the cell, but rather overwhelmingly or exclusively for the increase for regeneration of plasma products (cartilage or bone ground substance, muscle and nerve fibrils, etc.), then there is very slow or even no cell division at all. Therefore, the phenomenon becomes apparent that with increased histologic differentiation, body growth reaches a final point. 3) If the nuclear plasmic tension is not completely resolved by the act of the division, then there is another division immediately thereafter followed by a third, a fourth, etc., until the normal nuclear plasmic relationship is achieved. Thus the phenomenon of the reproduction process can be explained. At the beginning of this, there is a high degree of nuclear plasmic tension and an enormous faulty relationship between nuclear and protoplasmic mass. If with every division the nuclear material grows approximately to double its apparent mass at the beginning of division, then it requires indeed many divisions following one on the other, before the faulty relationship between nucleus and plasma is equalized and thereby the end of the reproduction process is achieved. If the cell is to divide still further, then it would require growth by means of nourishment. I might mention in passing that Morgan -- which was unknown to me at the time I formulated my conception -- developed a similar interpretation, that the end of the reproduction process is achieved if the faulty relationship between size of the reproductive nucleus and the ova size is equalized and the normal relationship of nuclear size and protoplasmic mass is achieved by means of successive divisions. He temporarily lost sight of this correct concept, but returned to it in his later publications. Also, Driesch supported Morgan's interpretation.

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If we review the discussion up to this point, we can view the theory of nuclear plasmic relationship as correct, i.e., that for a certain cell form,

there is a constant size relationship of nucleus and protoplasmic mass and that the cell size is determined by the nuclear size. In contrast, it is necessary to prove the concept in which I have tried to explain causally the nuclear-plasmic relationship via the interplay of two processes, functional growth and divisional growth of the nucleus. In earlier publications, I have already referred to this, and at the same time envisioned the possibility that this proof could be brought to a high degree of certainty by means of exact description of changes which the nuclear-plasmic relationship experiences in Infusoria from one division to the next division. At that time, I entrusted to one of my students, v. Wierzbizki, the task of constructing growth curves of the nucleus and protoplasm for the period from one division to the next in a subject very well suited for these measurements, Frondonia leucas. Since reliable mass data can be obtained only from dead material, the growth curve of the nucleus cannot be obtained by use of a single individual. Likewise, it is hardly possible to obtain the necessary data for a growth curve by killing many animals after division at earlier or later intermediate stages and entering the absolute sizes obtained from them directly into the curves. For this, there are bases, about which I will speak later, which allow separation of the division sizes of individual Frondonia. Therefore, I suggested to Mr. v. Wierzbizki that he measure relative masses: After division, which of course is essentially equal, the one animal is killed immediately and nuclear and plasmic masses are determined; the size increase which nucleus and plasma of the second animal experienced in each case after 4, 6, 8, etc. hours were converted to multiples of the mass which the sacrificed animal had yielded, and these relative determinations of size were used to construct a curve.

I had expected, on the basis of my conceptions of the functional and division growth of the nucleus, that the total body of Frondonia would experience a gradual increase from one division to another, that the nucleus, in contrast, would increase very little (functional growth), but during the division phase would grow rapidly in contrast (division growth). In fact, it became clear from the investigations of Wierzbizki that, while the Frondonia grow from one division to another, the nucleus experiences a very slight increase over a longer period of time and the necessary energetic nuclear growth for the production of a normal nuclear-plasmic relationship occurs only at the time during which the division begins and comes to completion. In general, my predictions were borne out; of interest was the fact that a new phenomenon occurred; that after the course of each division there was at first a diminution of the nucleus; apparently, at this time, the functional growth of the nucleus regresses in favor of renewed assimilatory activity. In fact, the size of the nuclear resorption corresponds to the size of the functional nuclear growth.

My investigations on protozoa have led to the further result that the nuclear-plasmic relationship is subject not only to periodic changes caused by rhythm of life phenomena, but by certain influences it can experience inhibitory changes. Such influences included: 1) uninterrupted function, 2) hunger, 3) change of temperature.

1) If protozoa are fed uninterruptedly and so carry out uninterrupted assimilatory activity, then the nuclear size will grow in the course of several weeks in overwhelming fashion at the cost of the protoplasmic mass, apparently,

because the reduction of nuclear mass during division is insufficient. The increase in the nucleus leads to an increase in the division size. With further activity, circumstances of functional noncompetency appear in the cell (cessation of feeding and division) which I have already described for the various protozoa (paramecia, dilepti, actinospheria) and for which I have taken the very apt expression of Calkin, "depression," and which I will retain in the following. Many protozoa are destroyed in the course of depression, others remain the same, specifically those which are successful in reducing the nuclear mass and becoming competent to assimilate. In such a way, a new energetic assimilation and growth period occurs. The more depressions a protozoa culture must withstand, the greater becomes the danger that the depression circumstances will be able not only to act in a regressive fashion, but that finally the culture will die out.

A development which I have described here should be called an autogene, because the cell of and by itself continues to form its life forces, and is distinguished from the amphigenic development, in which development experiences a modification by the fact that in Amphimixis, a merger of two cell organizations, occurs temporarily through reproduction. If we generalize all of our experiences, on the basis of protozoa, to all processes of cell life, then we can say that continuous autogenic cell development which is characteristic of vegetative (asexual) and parthenogenic reproduction, leads to a disturbance of nuclear-plasmic relationship, because nuclear substance increases too much at the expense of the protoplasm.

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2) If protozoa are subjected to long-standing hunger, then they may either encyst or, on the other hand, they may deteriorate gradually with an equivalent atrophy of nucleus and protoplasm, or thirdly, they may experience what I call hunger divisions. In one case which I followed very closely (in *Dileptus*), there were two rapidly-successive hunger divisions; in a case investigated by my student, Mr. Prandtl, with *Didinium nasutum*, the number of hunger divisions appeared to be equal. According to Maupas's results which were obtained a long time ago and therefore would require reconfirmation, the number of "hunger divisions" should be different for the different varieties. It was striking to me that starving protozoa have disproportionately large nuclei. I had my student, Mr. Kasanzeff, study these relationships very closely. It was determined from these studies that paramecia formed by hunger divisions are significantly smaller than those paramecia which reproduced under good nutritional circumstances, and that, in contrast, the nuclei of the first group are not only relatively but even absolutely larger than the nuclei of the latter. Hunger divisions rest therefore on an alteration of the nuclear-plasmic relationship in favor of the nucleus; the nucleus gains the authority to take material from the protoplasm again, without the latter having experienced any increase in its mass.

Since Infusoria, which are subject to hunger divisions, progress to conjugation, there appears to be a positive relationship between both phenomena. The hunger divisions are in agreement from this point of view with the maturity divisions of multi-cellular organisms, which has led me to the interpretation that there might be even more general agreement between these kinds of division, such that maturity divisions would actually be a category of

hunger divisions, an interpretation which I will have recourse to in the following discussions. The suggestion that the hunger divisions especially in Infusoria gain simultaneously the significance of preparatory divisions for sexual maturity encounters only one difficulty, that true maturity divisions and true spindle-body formation have been demonstrated for Infusoria; the divisions which occur after the start of conjugation and the partial regression of the nucleoli. Thus we would be inclined to characterize thereby a double maturity rather than a single maturity as would otherwise be the case. I suspect that this double maturity is a consequence of the duplication of the nuclear apparatus. First, the large main nucleus matures, and then secondarily, the nucleolus participates. In favor of this suggestion is the fact that the maturity processes are interpreted as necessary resultant phenomena of previous life processes, as processes whose course brings about reproduction, not only as processes which have the goal of preparing the nucleus for reproduction.

3) And in the final analysis, we must examine the influences which change nuclear-plasmic relationship, specifically the effects of temperature. I have established and it has in the meantime been confirmed by investigations in the Munich Zoological Institute, especially by the investigations of Mr. v. Wierzbizki, that increase in temperature reduces the nuclear size, while decrease in temperature yields an increase in its size. The diminution of the nucleus, moreover, results in that the nuclear-plasmic tension which is necessary for division is achieved earlier and the division size of the animal increases significantly. Infusoria become smaller when cultured in heat these smaller animals, however, have not only absolute but relatively smaller nuclei than animals cultivated at usual temperatures; their nuclear-plasmic relationship is displaced. Conversely, there is an increase in cold not only of the absolute nuclear size and as a result of the body size, but the nuclear-plasmic relationship changes as well.

It is appropriate at this time to refer to an interesting analogy which exists between the effects of cold and the effects of continuous autogenic culture which in both cases results in the growth of the nuclear-plasmic relationship. I have already explained this analogy earlier by means of the interesting encystment processes of Actinosphaerium Eichhorni. Actinospharia which are cultivated for month-long periods in a super-rich nutritional environment have an overwhelmingly increased nuclear count. Thus, at encystment, they have too many nuclei, even after a large part of the nuclei have been dissolved in the usual fashion. This results in very many mononuclear primary cysts being formed with correspondingly smaller size. If the cyst sizes in the first weeks of a culture are compared with those obtained after a month of continuous culture, then the cysts obtained at first are eight times as large as those obtained later. It may be concluded that there is an eight-fold relative increase in the nuclear mass and, in fact, even larger. For in continued culture, the nuclei of the smaller cysts are absolutely larger than the nuclei of the larger cysts at the beginning. Nuclear hypertrophy increases finally so much that it makes a normal run of the encysting process impossible and the encysting Actinospharia decompose into brown pigment masses. I have already reported this characteristic increase in the number and diminution in the volume of Actinospharia cysts and suggested that in this way the maturity divisions take an abnormal course and can acquire the character of masculine maturity

divisions. Since that time, I have been able to confirm my observations on extended further cultures (in the winter of 1903-1904); I always sought to obtain diminution in the size of cysts and disturbances in the maturity divisions by means of long, continuous culture.

Experiments carried out by Mr. Smith under my direction have shown that also in cold cultures the Actinospharia produce many small cysts with increased chromatin content in the nuclei. The change obtained this way in the nuclear-plasmic relationships, however, was not as significant as the change resulting from long continuous autogenic development.

The reason that I have discussed the meaning of the nuclear-plasmic relationship and those influences which modify the nuclear-plasmic relationship in such great detail is clear, if we observe that there is a large difference between the mature egg and the mature spermatozoon with respect to nuclear-plasmic relationship, which is found in no type of unicellular animal. Ova nuclei and spermatoc nuclei contain, as we have found from investigations on fertilization, an equal amount of nuclear substance, while in contrast, the egg is enormously rich in cell mass, the spermatozoon has very little. The nuclear-plasmic relationship, therefore, is changed in mature sexual cells in comparison to other cells and indeed in both sexes these changes are extreme opposites; the same is true for the macro- and micro-gametic single-cellular plants and animals.

The characteristic change of the nuclear-plasmic relationship in sexual cells gives a better understanding of their function in fertilization and the embryonic development which follows fertilization. In order that fertilization and embryonic development can take place, there must be fulfillment of three conditions: 1) That the sexual cells can come into contact easily, 2) that the zygote has enough mass for development, 3) that amphimixis occurs, i.e., union of two equal-sized nuclei from different individuals. These three pre-conditions can be achieved only when for the sake of preservation of equal nuclear sizes the protoplasmic content is markedly reduced in one case in the interest of increased mobility, while in the other case it is increased in the interest of favorable nutritional conditions. It is in this division of labor of the cells with maintenance of nuclear quality and in the change in nuclear-plasmic relationship which is directly tied to this change that we see the only general recurrent criterion of sexuality known to us at this time. We have presently no reason to assume that sexual differentiation is based on any further, more fundamental causes; however, we do have reason to assume that all of the differences have developed in connection with the above-mentioned differences in cell regulation, which cause such striking differences of masculine and feminine individuals in so many cases.

If we make use of the observations obtained in the discussion of the sexuality problem, i.e., the question of the determining factors of sex, then we must seek all causes which influence these factors which conversely have an effect on the nuclear-plasmic relationship. We have learned some of this by means of experiments on protozoa; investigations included autogenous development, effects of hunger, influence of temperature. In the last few years, I have begun partly on my own and partly with help of my students, to investigate

the significance of these factors for the sexuality problem. It is concerning these experiments that I wish to report here.

II. The Present State of the Sexuality Problem

First, I would like to make some introductory comments about the present state of the sexuality problem. I think this can be best characterized if I designate the following as the predominant interpretation, that the sex of progeny is determined exclusively by the mother, that the sex is determined by processes in the egg even before fertilization, that there are specifically masculine and feminine eggs, i.e. predestined for masculinity or femininity. This interpretation has been supported recently with great certainty by Lenhossek, Bernhard Schultze, as well as, if somewhat less decisively, by Oscar Schultze, Beard, and others. This point of view derives from several very marked facts which, however, have been over-emphasized regarding their value in the proof, as well as by the lack of success in determining sex with external influences; this point of view is possible only if the remarkable fact which is not contested even by the opponents of the Dzierzon-Siebold theory, that unfertilized frog eggs always produce males while, in contrast, fertilized ones yield females (according to the predominant interpretation, exclusively, according to Dickel, only overwhelmingly), is ignored. Or, the viewpoint would be possible only by bringing willfully untenable hypotheses into harmony with the prevailing interpretation. /195

With respect to the newer kinds of interpretation of the sexuality problem which are not favorable for the development of research, I would like to emphasize that the problem does not stem from a single source, according to the interpretation which I gave in the introductory comments, neither with reference to the sex-determining factors nor with respect to the point in time of sexual determination. Only one certain form of cell regulation is common to all forms of sexual differentiation. The causes which underlie this cell regulation can be of very variegated nature, in contrast. Autogenous and amphigenous development, the latter proportional to the difference in the sexual cells, influences of nutrition and temperature, and perhaps others which are unknown to us, can modify these regulations. Often there is a case of influences in the same direction and in other cases in opposite directions by means of competing effects of the above-named factors, so that sex finally would be the result of various sex-determining factors. With reference to the problem of the period of time of sexual determination, it is sufficient to refer to the existence of hermaphroditic animals in order to show that in many cases the fertilized egg is not sexually determined. It would be necessary to come to the arbitrary decision that the fertilized egg itself is a hermaphrodite, and thus is not fully differentiated, which event first occurs in the course of development. As of now it can be said only that the fertilized egg has a certain constitution which under usual conditions will produce either a masculine or a feminine or a hermaphroditic organism. In contrast, it is not yet certain whether an egg predestined for one sex by certain conditions of further development can not be altered by other strong influences to bring about another sexual result. In fact, I think this is even more likely and I am able to introduce reasons for this into the discussion, even though it would seem that the degree of sexual predetermination is variable according to the organisms.

If we penetrate deeper into the sexual problem, we will be able to see in detail, presumably, what at present is apparent only in gross detail, that the sexual character of an individual is determined at very different periods of time, very late for hermaphrodites, among whom sexual differentiation begins only within the sexual glands, while in other cases, in the freshly-fertilized or even unfertilized egg. The earlier sexual differentiation appears, the more the specific sexual character is expressed in all cells of the body, so that in many cases all tissues carry the stamp of the sex in more or less noticeable ways. What is needed, perhaps, is only refinement of the research methods, perhaps even only the correct questions remain to be asked in order to understand this problem in detail.

How did this interpretation find such wide acceptance, that is, that sex is always already determined in the unfertilized egg? In my opinion, this is easily understandable, for it is necessarily derived from this that the dominant influence must be ascribed to the egg, even if not decisive in all cases, it indeed must be such, that under certain conditions, it alone is decisive. The following considerations will make that clear. The specific nuclear-plasmic relationship, in my opinion, is sex-determining. This depends on the mass and the activity of the nucleus, and that of the protoplasm. Now the egg yields all the protoplasm for the young embryo and half the nuclear substance, while the spermatozoa, in contrast, bring only half the nuclear substance. Thus, it is the egg which determines the lion's share of the nuclear-plasmic relationship, insofar as it supplies all of one group of sexuality-determining factors and half the other. There will be cases in which the egg has a striking reduction in protoplasmic quantity and therefore, such an energetic regulation of the nuclear-plasmic relationship toward the masculine direction that the quality of the spermatogenic nuclei no longer matters in the balance. This is especially relevant for eggs which are always used as proof for the ovarian determination of sex, e.g. for eggs of Dinophilus apatris, in which the masculine eggs are rudimentary, and about one-sixth as large as the feminine eggs. In the last analysis, from what we know of fertilization, the nuclei of these small eggs must be just as big as those of the larger eggs; the different sizes of the eggs, therefore, must influence the nuclear-plasmic relationship in an energetic way and thus the sex.²

That there are also cases in other animals in which the size of the egg has such a sex-determining effect is apparent from observations of insects. Brocadello and Joseph were able to determine for Bombyx mori and for Ocneria that in sorting of nest into larger and smaller eggs, 88-92% of the smaller eggs yielded males and 88-95% of the larger eggs yielded females.

²Such small eggs develop into rudimentary males, of very small body size and without gut. I believe that this is the consequence of excessive masculine differentiation of the egg. If the plasmic mass of an egg sinks below a determined amount, then inhibitory factors must come to the fore. Then, after this, the latter comes to an early standstill, which we became acquainted with, via the causal complex of nuclear-plasmic relationship and fertilization process; therefore, enough material for the development of all organs and above all for gastrulation cannot be supplied, and thus rudimentary animals arise.

Very significant size differences are found among the eggs of the frog, sometimes within one nest and even more often between eggs of different nests. In Rana esculenta, to offer an extreme example only, I found the eggs of one female to be about 1.1 mm, while those of another were 1.8 mm. My experience with frogs which I will come to in the following, emphasized however that care must be exercised in coming to conclusions on sexual predestination from the size of eggs only. The egg sizes, as well as cell sizes, are caused by still other influences, e.g. temperature. There are eggs which must be placed at low temperatures for development and other which must be placed at higher temperatures for development.

In many cases, perhaps in the majority, the eggs of a single species appear to have the same size; these are found to be in a kind of labile condition with respect to their sexual tendencies. In such cases, the participation or exclusion of the spermatogenic nucleus (parthenogenesis) and in the first case the activity of the spermatogenic nucleus can be sex-determining. A certain example of this, I think, is the situation with frog eggs which was discussed above.

III. Experimental Investigations

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1. Daphnids

I turn now to a discussion of experiments performed in the Munich Zoological Institute and begin with the investigations on daphnids. With respect to these organisms, Weismann, in his epic-making investigations, is believed to have proven that they have a cyclic propagation which could not be altered by means of outside influences. According to his information, a certain number of parthenogenetic generations follow one upon the other before a sexual generation of male and female occurs. The number of parthenogenetic generations forming such cycles is standardized for the individual types in different ways; types which are exposed to frequently-recurring damage are polycyclic, and have several cycles in one year including only a few parthenogenetic generations; other types are monocyclic and produce very many parthenogenetic generations, therefore the once-yearly sexual generation develops. This regulation of propagation probably formed as a result of contact with conditions of existence which offered certain challenges, but probably became independent gradually from the external conditions of existence and have been maintained as internal processes of the organism.

Weissmann's results have been challenged repeatedly; it was repeatedly claimed that the appearance of males was a result of poor nutrition just as Hydratina and Hydra according to Nussbaum show formation of the male sex by means of hunger. Since the work available at the present time did not appear to me to be very convincing, I had my student, Mr. Issakowitsch, investigate anew the sexual relationships of the daphnids, chiefly in regard to the effect of temperature. Specimens of Simocephalus vetulus were used in the experiment which appeared suddenly in large numbers in a culture container which I had observed for a long time, which had either developed directly from Ehippia or represented at the most a second or third parthenogenetic generation of a cycle developed in the winter. Innumerable cultures were set up by Mr. Issakowitsch, some at 24°C some at 16°C and some at 8°C, all receiving abundant

nutrition. Furthermore, the progeny of a female raised in warm culture were repeatedly divided into two equally strong cultures and one half continued in warmth, the other half, in some experiments, at room temperature, in others, in cold. The results concerning which Mr. Issakowitsch will report in detail were very unambiguous. The warm cultures yielded several generations following one after the other of parthenogenetic females, only here and there a single isolated male. The cultures exhausted themselves finally and died out.

With daphnids cultured at 16°C or 8°C or carried into these temperatures from the warm room, there was formation of sexual generations, immediately in the cold culture, at 16°C only after another couple of parthenogenetic generations had appeared. The further broods of the mother animals transferred to cooler temperatures also changed their character: The first brood consisted of parthenogenetic females still; later, males and winter eggs appeared. The succession of the latter two kinds of eggs was very interesting; first, in general, eggs formed which yielded males, and only after that, winter eggs, although winter eggs were able to develop immediately after transference into the cold. From these experiments it can be seen without doubt that the cyclic propagation of daphnids is changed by temperature influences, in that lowering of temperature first yields males and after further lapse of time, the formation of winter eggs. Whether this is a question of direct influence of temperature on the sex cells, which I consider probable, or whether the temperature is of importance only insofar as it changes the nutrition conditions, as Mr. Issakowitsch assumes on the basis of certain observations and considerations which he will report later, it is by no means a question of purely temperature or hunger effect, but as the cultures at medium temperatures show (16°C), the activity of the sexual apparatus is of importance. Continuous parthenogenesis is an autogenous development and in this sense comparable to the asexual propagation of protozoa. If several parthenogenetic generations follow one after the other, then experiences obtained with protozoa can be expected, that if autogenous development permits the nuclear mass in the cells to experience a gradual increase, then the nuclear-plasmic relationship will suffer a modification toward the masculine direction. Perhaps this transformation of the cell can be suppressed for an extended time by means of an opposed effective factor such as warmth. If, in contrast, the effects of autogenous development join the similarly-directed effect of lower temperature, then masculine eggs arise. The fact that, at striking reductions in temperature, males are formed immediately, could serve as proof that the temperature, as long as it is sufficiently lowered, fully sufficient to evoke a condition attainable otherwise by a combined effect of internal and external forces.

The explanation that I gave now appears to contradict, that a further increase of the factors which alter the determination of the sexual apparatus yields female eggs anew, which produce winter eggs. For, from the fertilized winter eggs, demonstrable females arise which are capable of parthenogenetic development. It should be considered here, however, that the winter eggs arise in another fashion than parthenogenetic and masculine eggs. While the latter arise from a fourfold group (one egg + 3 abortive eggs), a larger number of fourfold groups unite to produce a single winter egg. This is reminiscent of the depression circumstances of Infusoria during which most animals go to

their destruction as a result of nuclear hypertrophy, while others recover with reduction of nuclear size. I would like to compare a persisting fourfold group to a self-regenerating Infusorium: The fourfold group, which is becoming a nutritive cell, to Infusoria, which are being destroyed by depression. When these different kinds of fourfold groups unite with each other, an egg arises with rich protoplasm and an undeniable nuclear-plasmic relationship.

2. Experiment on Dinophilus apatris

The theories gained from observations of daphnids are appropriate to clarify the findings which Mr. v. Malsen has obtained. At my request, he investigated the effect of changing temperatures on the sexual generation of Dinophilus apatris in the Munich Zoological Institute. According to the elegant experiments of Korschelt which have played a large role in the discussion of the sexuality problem in recent times, two kinds of eggs are raised in one nest by Dinophilus apatris, very small eggs, from which the rudimentary males arise, and very large yolk-rich eggs which develop into female animals. If Dinophilus is cultured at a temperature of 10-12°C, then the proportion of male to female eggs is 1:3, a ratio which is derived from months-long culture, and counts of several hundred eggs. Warm cultures (25°C) yield, in contrast, a sexuality ratio of 1:1.75, which rises occasionally to 1:1. Completely contrary to my expectation, it turned out also, as Maupas had also shown for Hydatina senta, that warmth favors the formation of male eggs. In order to understand this behavior, a close investigation of ovogenesis is necessary. This shows that the eggs of Dinophilus, in similar fashion to those of the daphnids, arise from the union of several egg primordia. The size of the eggs depends on the number of uniting egg primordia. Just as in winter eggs of daphnids, the count of cells uniting to form one female egg appears extraordinarily larger than in male eggs, which again allows the conclusion that it is a question of a higher degree of "depression" as is favored by cold. The lack of small parthenogenetic eggs prevents drawing a parallel with the relationships of Dinophilus and daphnids.

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3. Experiments on Frogs

The experiments referred to heretofore allow indubitable recognition that temperature exercises a very significant influence on the sexual relationships of animals; they suffer only from the unfavorable circumstance that by means of it no direct influence of the egg is achieved, but an influence which has an effect on the organism of the mother animal and gains influence on ovogenesis.

It occurred to me at that time to ameliorate this unfavorable circumstance by choice of a favorable experimental subject and so I extended my experiments to frog eggs which have already played an important role in this discussion of sex-determining factors.

a) Influence of egg maturity on sex. The suitability of the subject was the reason that I did not limit my experiments to temperature, but rather investigated as well the influence of various degrees of maturity on the sexual determination. The following considerations stimulated the latter

means of investigation. The egg grows to a significant size under the dominating influence of the follicle on metabolism, before egg maturity occurs, and thereby any further increase in the size of the egg becomes impossible. If it were now possible to bring about a premature maturity in the egg and thus bring egg growth to a standstill, then perhaps a nuclear-plasmic relationship favorable to the formation of male animals similar to that of the rudimentary eggs of Dinophilus could be achieved.

Many things favored the fact that egg maturity could be stimulated by a hunger condition of the egg, an interpretation at which Lebrun arrived too, completely independent of me, in his experiments on amphibia. In one case, this was favored by the above-mentioned experience that the mature divisions of Infusoria are hunger conditions. Further support was offered to my suspicions by the circumstance that the egg maturity is brought about by dissolution of the follicle. Nuclear dissolution occurs mostly, however, as a result of hunger conditions, as my investigations on protozoa especially heliozoa and radiolaria have taught me. A third hint was suggested to me by the maturation of Asteracanthion eggs. This appeared if the eggs were emptied from the ovary and thus their source of nutrition was denied. As I have learned as a result of other experiments, maturity can be forced on eggs which are still distant from normal egg maturity by emptying them from the ovary, which then, to be sure, evolve very slowly in comparison to normal situations.

Helen King also produced egg maturity with amphibians by well-known methods in Asteracanthion, by taking the eggs out of their follicles in the ovaries into water. I did not consider this method, however, appropriate for my purposes since such premature eggs are not provided with the gelatin envelope that derives from the oviduct and therefore offer unfavorable conditions for fertilization and normal embryonic development. Thus I avoided this procedure even though I admit that it deserves to be tried. It would be conceivable that with careful treatment the lack of a protective envelope could be compensated.³

In nature, the ejection from the follicle and passage of the egg into the oviduct is stimulated by the embrace of the male. I am in agreement with Lebrun on the method of operation of this effect in assuming that circulatory disturbances in the ovary are brought about by compression of the vessels. I tried, therefore, for a long time to substitute for the effect of the embrace of the male before the onset of sexual maturity, an elastic ligature. In fact, I was successful in a large number of Rana temporaria in bringing about the transfer of eggs from the oviduct three weeks before the beginning of general sexual activity. For Rana esculenta I decided on a different method. I had a previous student of mine, Prof. Kwietniewski in Padua, whom I would like to thank at this time for his help, send males separated from pairing, from upper Italy, and permitted them to pair with Munich frogs, among

³Another possible means of obtaining eggs with a reduced nuclear-plasmic relationship would be reduction of the protoplasmic mass by means of puncture of the egg. It would be very interesting to attempt this. The unpredictability and difficulty of this procedure on the one hand and the lack of time on the other, however, caused me, after several unsuccessful attempts, to give up at present on this and to work on premature maturity.

whom rutting occurred significantly later in the previous year; I obtained in this way normally laid and fertilized, but prematurely mature eggs.

The fertilization of premature eggs both in R. temporaria and in R. esculenta led to very remarkable results. With R. temporaria I was forced to carry out artificial fertilization and thereby to use sperm from animals which did not yet show any sexual drive. In three such fertilizations, very few eggs appeared and of these an extremely small number developed further. From one fertilization, I obtained three larvae, and from two others, a total of 13 larvae, extraordinarily small numbers, if one considers that in the first case perhaps 1,500 and in the two other cases maybe 3,000 eggs were used. Somewhat better results occurred in the natural way from eggs laid by females and fertilized by males of Rana esculenta. One nest contained perhaps 3,000-5,000 eggs; nevertheless, only 60 larvae developed, and of these, 40 achieved metamorphosis. These 40 animals were, without exception, males. Also the three larvae of the first R. temporaria were males. Of the second temporaria fertilization, 13 larvae unfortunately died prematurely from an overwhelming glochidia infection because the laboratory assistant fed them in my absence with glochidia containing mussel gills.

From these investigations, it can be seen with certainty that eggs laid prematurely offer very unfavorable development conditions. The reason for this needs more exact investigation. Until now, I have had no time for this. It is very likely, furthermore, that premature eggs yield only male animals, for there is no reason to assume -- even if it is not possible to show such an assumption to be untenable -- that all eggs predestined to become female animals went to their destruction. The experiments done so far have turned out largely in favor of the interpretation which I entertained earlier; I encourage investigations in the suggested direction and of further methodical approach.

It was desirable to complete the studies on the sex-determining influence of prematurity since the investigations on the effect of postmaturity were extended. I was moved to do this by accident: the previous year a female esculenta was brought to me from the wild which was so overripe that the eggs came out spontaneously. I used first that morning the contents of one uterus and used the contents of the second uterus in the afternoon, therefore about eight hours later. The first fertilization turned out well; the second gave poor results in that, at the most, only half of the eggs gave rise to larvae capable of development. I would like to suggest, on the basis of many experiences which I have had in the meantime, that the result may be explained in the following way: that the eggs of the first fertilization had already achieved a high degree of maturity, and that further delay, despite favorable external conditions, introduced a disturbing factor into the capability of the eggs for development, i.e. postmaturity. I raised the overripe eggs in parallel with three other fertilizations which derived from normally-mature esculenta pairs, under varying temperatures; the eggs were, some from the beginning, some after several days, put into 22°C heat, and later up to 30°C for development, and in part they were subjected to changing room temperature, and part to cold (15°C). In order to feed them abundantly, I used a container of wire which was put into large basins, and allowed water to flow.

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through. Unfortunately, I must acknowledge high mortality in all the cultures, which must partly have something to do with the fact that Rana esculenta is more sensitive than Rana temporaria, partly as a result of the higher calcium content of our tap water. Unfortunately, I discovered too late that earlier experimenters, even as far back as Roesel v. Rosenhof, had become aware of the damaging effect of calcium-containing water. Only a small percentage of the attempted cultures developed to metamorphosis (20-40%), or, more exactly, to a stage at which sex could be determined with certainty. I mention this because these large losses impair the strength of my argument based on my results quite appreciably. If I add total male and female frogs which derive from the same pair, unconcerned with temperature at which they were maintained, then I arrive at the following relationships of male and female individuals from my cultures.

Premature eggs, Culture A	0 ♀	40 ♂
Normally-laid eggs, Culture B	90 ♀	78 ♂
Normally-laid eggs, Culture C	21 ♀	89 ♂
Normally-laid eggs, Culture D	84 ♀	189 ♂
Postmature eggs, Culture E	13 ♂	317 ♂ [sic]

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In this table, Cultures B, C and D are characterized as normal, as cultures which were permitted to develop at normal sexual maturity; they were also characterized in that the eggs were very similar. A is the already-discussed culture of premature eggs; E the postmature culture. Common to the two latter cultures is the fact that the fertilization was undertaken at a time when the optimum of developmental opportunity had not yet been achieved, or began to be exceeded; common to both also is the enormous preponderance of male character, insofar as in one case only males, in another, some females, but very few, were produced. Since all cultures were kept in the very same environmental conditions, it is extremely unlikely that the totally different sexual relationship in Cultures A and E in one direction and Cultures B, C and D in the other, could rest on the basis of selective destruction of one of the two sexes. Everything supports far more the fact that the very different sexual relationship is the consequence of the different stages of development of the eggs, that, at the height of the mating period there is an optimum for the female sex, which is altered in favor of the masculine sex the nearer the eggs are to the beginning or the end of the fertility period. This result is even more worthy of attention since it agrees with many experiences of animal husbandry. Also, the noteworthy phenomenon that the tendency of eggs to produce males falls near the borders at which their normal developmental capability ceases finds an echo in statistical experiences. It is a long-familiar experience that the mortality of male children is significantly higher in the first year of life than is that of females. Another statistical fact is relevant here. The usual human sex ratio is 105-106; i.e., for every 100 girls, there are approximately 105-106 boys born. In stillbirths, the sexual ratio climbs to 135, and in premature births, for which, to be sure, as far as I know the situation, a very sparse statistical sample is available, it is about 159-174%. These numbers favor likewise the theory that the situation of fertilized egg cells which becomes the jumping-off point for male development, is very close to the point where developmental capability is damaged or destroyed.

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Now, it would be relevant to place the above-mentioned striking predisposition of premature or postmature eggs to male development in juxtaposition with the lesson of nuclear-plasmic relationship. For the premature situation, I had expected even before the beginning of my experiment a male nuclear-plasmic relationship, because it is apparent that, at the critical stage, an insufficient formation of protoplasm has taken place. For the postmature eggs, such an explanation is ruled out, since there is no reason to assume that a postmature egg would have less quantity of protoplasm than an egg laid at the height of sexual maturity. In my opinion, the changes in this case could only derive from the nucleus, in that the latter had experienced an increase in its substance. I have formulated other concepts, the elucidation of which require further groundwork.

By means of my experiments first, and later by means of Loeb's experiments, it became clear that chemical agents could have an effect on eggs stimulating parthenogenetic development. Loeb took upon himself the great effort of bringing this method to such perfection that parthenogenetic development stimulated by means of reagents could lead to formation of normal larvae. According to my experiences with sea urchin eggs, a tendency toward parthenogenetic development occurs spontaneously even without reagent stimulation if the eggs are allowed to remain for a long time without fertilization in sea water, except that they soon come to a standstill. In contrast, among starfish, blastulae and gastrulae develop (C. Greeff, O. Hertwig) in these spontaneous parthenogeneses. Apparently there is a greater or lesser tendency toward parthenogenesis among all eggs and also among frog eggs. Since the latter arrive in the uterus at the stage of the first spindle formation, the tendency for completion of egg maturity with long-standing sojourn in the uterus would lead first, and perhaps later as well, to metabolic exchange between the egg nucleus and protoplasm, which must be shown first, however, by very precise experiments. The fertilization of postmature eggs would be in one sense the fertilization of eggs in which the first preparations for parthenogenetic development have already been introduced.

That parthenogenesis belongs to the sex-determining factors and indeed that it favors the male sex is demonstrated by the above-mentioned experiences with frog eggs which yield male offspring in the unfertilized condition. The significance of this fact is not altered by the fact that among other hymenoptera, and further, in aphids and daphnids, many parthenogenetically pure female generations follow one after the other, before males are formed. For there can be no doubt that the eggs of different animals are, with respect to sexual differentiation, regulated very differently. There could be eggs of equally labile sexual balance which would react quickly to sex-determining influences, on the other hand, eggs or egg generations which are predisposed toward one direction, be it toward the female or toward the male, which could be transformed only by means of very energetic or very frequently-repeated influences. I would like to offer frog eggs as an example of labile sexual equilibrium, while in contrast the eggs of aphids derived from winter eggs and of daphnids, in contrast, are eggs with a strong impression of female character. There must be a situation where several, and in some cases, very large numbers of generations must be subjected to the influence of parthenogenesis and connected with that, the transformation of the nuclear-plasmic relationship by means of autogenous

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development, before the cumulative effect is sufficient to cause the appearance of male animals.

I believe that the discussions given thus far are sufficient to make understandable how I have tried to explain the favoring of the male sex by overmaturity of eggs. From this, certain consequences are derived, for the evaluation of variable relationships between fertilization and sex-determination, which I will discuss in further detail.

If parthenogenesis favors the development of eggs toward the male side, then it is necessary to assume that the fertilization is suitable to reinforce the tendency toward the female. Thus, the sex problem comes into closest relationship with the fertilization problem with the question: What is the physiological task of fertilization?

Most zoologists lean at present to the interpretation of Weismann concerning fertilization, according to which fertilization fulfills the task, by means of amphimixis, i.e., by manifold mixture of characteristics of the parental pair, to evoke a totality of forms from which the natural breeding selection allows the most suitable to endure. The fertilization process would therefore be a purposeful orientation of the world of organisms, not a necessary resulting phenomenon of the life processes; in this case, it could not be predicted how fertilization might bring about sex determination, and indeed always in the same sense.

In contrast to the Weismann interpretation, it was assumed earlier that fertilization and indeed fertilization by organisms which were not very closely related ("rejuvenating the blood") would be necessary to create a favorable development of the progeny. This interpretation which holds fertilization to be a physiological necessity, which, however, would not exclude the theory of amphimixis as a means of breeding selection, is the interpretation which I maintain is correct. Just as autogenous development over a long time, in protozoa, leads to degeneration and depression, so would a similar situation be relevant for multi-cellular organisms. It has been shown that there are plants which propagate for many centuries vegetatively by means of sprouts, without showing any damage, and it can be concluded from this that the damaging effect of autogenous development is not so bad, that fertilization cannot have the goal of counteracting this damaging influence. I cannot agree with this line of reasoning; I believe, moreover, that in all the cases cited in which nature has likewise abandoned fertilization, the healthy effect of fertilization is replaced in the organism by other arrangements. We recognize in protozoa some temporary arrangements of spontaneous cell reorganizations and likewise with the cell reorganization involved in an encystment. Furthermore, the dormant stage of plants, e.g. the winter rest, will supply a substitute for the effect of fertilization and make the latter expendable to a great degree.

The opinion that fertilization is a phenomenon deeply rooted in the essence of the organism has also received essential support by the proof that fertilization is quite widespread among protozoa as well. I believe that we will soon be able to say that it is generally the case. Of great importance

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moreover, is the means of distribution and the frequency of the fertilization process in the individual groups of organisms. It is quite clear that the higher the organization of an animal is, the more lively its functions take place, and the more the fertilization process becomes an indispensable necessity and every propagation is bound up with sexual processes.

If we now attempt to form a more exact idea of the beneficial effect of fertilization on the organism, then we must mention first that experimental investigations on protozoa have led to the very definite result that the fertilization cannot have the goal of providing the organism with the ability to divide which was once lost to it. Therefore, I have suggested the hypothesis that fertilization might be a regulatory process; the introduction of a foreign element, such as the spermatonic nucleus, might retard an over-enthusiastic development of the cell activity. As in excessive growth of the nuclear substance in protozoa which becomes a cause for the fact that fertilization processes are destroyed, so conversely, fertilization might be a means which opposes the functional nuclear hypertrophy which would be damaging to the organism.

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This hypothesis would not only offer the advantage of explaining the damaging results of autogenous development, but also of inbreeding; it would also explain how fertilization can have a sex-determining effect. We could then expect that the sex-determining effect of fertilization will have the more of an effect on the development of the male, the more differently organized and the more effective the spermatozoon is in comparison to the egg. The more similar egg and spermatonic nuclei are, which occurs in inbreeding, the more favorable will be the course of fertilization for the production of males, which is in general agreement with the experiences of animal husbandry. On the other hand, a diminution of the effect of the spermatonic nucleus can be brought about by the fact that its substance loses vital energy or mass. I choose to ignore the first point which is known to play a large role in sex statistics; in contrast, I would like to bring the reduction of nuclear mass into the discussion, since this has been shown for the spermatozoa of many invertebrate animals (mollusks and arthropods) and most precisely for Paludina Vivipara. The total effect of such chromatin-poor (oligopyrene) or chromatin-free (apyrene) spermatozoa is completely puzzling. The circumstance that the apyrene and oligopyrene spermatozoa appear in numbers equal to the eupyrene makes the suspicion expressed on many sides that they might not be used only for fertilization, highly unlikely. With respect to this, a student of mine who has been studying this remarkable relationship, Mr. Popoff, is convinced, in contrast to earlier authors, that both kinds of spermatozoa, although not always, are found so frequently in the receptaculum seminis and at the upper end of the oviduct, i.e., at the place where it is expected, that fertilization of the eggs is completed. Let us assume that apyrene spermatozoa are used for "fertilization," then this would be not a true fertilization, not a union of paternal and maternal nuclear portions but rather a development stimulus; in principle, it would be in agreement with the process of parthenogenesis. The incomplete fertilization with oligopyrene spermatozoa would then be on the border in its effects between parthenogenesis and true fertilization. All of this makes the suggestion likely that the remarkable differentiation of spermatozoa is linked to sex determination, that the "fertilization" with

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apyrene and oligopyrene spermatozoa has the task of producing males. The sex-determining ability which is a function of the female in the formation of large, yolk-rich and small rudimentary eggs would in this case be transferred to the male sex.

b) The influence of temperature. Since I have studied the effect of temperature and propagation relationships of protozoa myself and also had directed Mr. Issakowitsch and Mr. v. Malsen to study sexual differentiation in invertebrate animals under the influence of changing temperatures, it was convenient to maintain frog cultures at warm, cold, and room temperatures. I have performed such experiments with Rana temporaria and Rana esculenta last year, and continued them this year. I am not very satisfied with the results obtained in these experiments. The problem here is very complicated, much more than it appears at first glance. Difficulties which I cannot go into here in detail, arise concerning the proper selection of temperatures; further difficulties relate to the time period, in which the effect of especially high or especially low temperatures should begin. According to my present experiences, it appears, for example, not to be immaterial whether the eggs are exposed at the time of fertilization or during embryonic development or during post-embryonic larvae stages, to the influences of elevated or reduced temperature. To the best of my knowledge, the egg has a high degree of adaptability during the fertilization process to changed environmental conditions, which is not present in equal degree later.

Also, the size of the egg, which I found occasion to discuss earlier, represents an important factor which demands attention. It has been known for a long time that the size of amphibian eggs varies extraordinarily. I found in Rana esculenta that several female eggs had a diameter of 1.1 mm, others, 1.8 mm; similarly, this is true for Rana temporaria, whose eggs are larger on the average than those of Rana esculenta. When I discussed the significance of egg size earlier, I referred to the explanation which is now relevant, that the size difference is bound up in sexual differentiation, that smaller eggs might yield males, larger eggs, females. It is likely that such a claim ought to be restricted to size differences within one and the same nest, although the necessary proof would first have to be provided in the case of frogs. The size differences between eggs which derive from different individuals are in contrast certainly the results of individual variations, which have subsidiary significance for the sexuality question. I am inclined to put these differences which arise from individual to individual in context with the extremely variable temperature conditions under which frogs mate.

Based on what I have learned concerning the correlation of nuclear and cell size on the one and the temperature of the environment on the other, eggs of different sizes each according to temperatures in which they exist are influenced in their development either positively or negatively. I believe that the variable egg sizes are likewise a kind of insurance against different temperatures. Smaller eggs with larger nuclear-plasmic relationship I would consider cold eggs, and vice-versa. I have come to this assumption because I have repeatedly made the observation that in one culture which was fertilized at moderate temperature and half of which was gradually introduced to cold and the other half transferred to warmth, the halves behaved completely differently. At first, high mortality predominated in warmth and in cold, mostly

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in the former. In a culture which was taken from the wild, and unusual for the small size of the eggs of Rana temporaria, all larvae died in the warmth (22°C) before or after hatching, while, in contrast, all in cold survived. This culture appears, based on what can be said so far, despite the small size of the eggs, to produce a very extraordinary percentage of females. I have made similar observations with Rana esculenta as well. The mating time of Rana esculenta occurred this time after a long, unusual cold period with a rapidly-increasing temperature. It can be seen from this that, of two nests fertilized at room temperature and cultivated, whose eggs had an unusually small size of 1.1 and 1.3 mm, that one did not develop at all while the other did not proceed beyond the stage of gastrulation. At that time, the temperature in my room was unusually high.

Parallel cultures in warmth (25°C), in lower temperatures (with a mean of 16°C) and in the cold (13°C) were set up with Rana temporaria. I had very large losses in warmth and cold, so that from an initial material of 200 animals, only 67 and 30, respectively, could be cultivated further; in contrast, I was able to bring 122 animals to metamorphosis in the room. New difficulties arose in sex determination. Of the 67 warm animals, I was able to determine four as males with fair certainty, and all the others as females. Of both other cultures, I got the impression during examination with a magnifying glass that an enormous percentage and in the cold, in fact, all, were males. However, on investigation of sections, I found in many animals which I had called males, varying amounts of eggs, so that I became very uncertain of my sex determination, whether I was dealing with testes containing scattered eggs or ovaries inhibited by temperature. Without doubt, the development of the sex is disproportionately more inhibited by cold than the development of the other organs. I must therefore consider this first experiment with Rana temporaria to be a failure.

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In Rana esculenta, it is very easy to determine the sex at a relatively early stage of development, so that from this standpoint I must consider this animal an excellent subject. Here, however, I had, as mentioned above, very extraordinary losses in the culture. In general, I had used too low temperatures, so that my warm cultures (at the beginning, 22°C, later up to 26°C, finally, 30°C) perhaps corresponded to natural temperatures, the middle temperatures (18-21°C) must count as cool temperatures. The cold culture (at the beginning 13°C, later, 15°C) died out almost completely.

When I put all of the animals kept in warmth until metamorphosis in groups according to sex, I obtained 127 females to 245 males, which is a relationship of almost 1:2; if I similarly compare those in cooler or colder culture, then a relationship of 1:5 occurs (54 females to 282 males). Now it seems desirable to ignore the postmature culture which had yielded an overwhelming number of males; the counts then are altered for the one culture to 121:133; for the cold culture, to 47:92. In the first case, the relationship would be practically 1:1, in the second, closer to 1:2. In general, these counts suggest that the warmth favors the production of females. Unfortunately, the counts are too small for statistical results. I would attribute to them even less significance if the daphnids and Dinophilus results did not show an indubitable influence on sex by temperature.

I have continued the temperature experiments this year and will repeat them in the succeeding years and expand them to warm-blooded animals. Many perhaps will not believe in favorable prognoses for these latter experiments, since the warm-blooded animals are supposed to be independent of the environmental temperature by means of their own temperature maintenance. Relevant to this is a phenomenon that the sexual maturity in warmer climates occurs at an earlier age than in colder climates, which supports the idea that the sexual apparatus is influenced by external temperatures.

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Addendum. During this year, I have carried on the experiments which I began last year, with new material, first with Rana temporaria and then with Rana esculenta. I have especially emphasized the culture of postmature eggs. I obtained these this time by separating pairs and undertaking fertilization as the female, despite maximum desiccation (i.e., moisture, but without water in the container) began to lay eggs spontaneously. In this case as well the mortality rate was high, in part even at very early stages of development, so that not even half of the eggs of one female, which retained the eggs for a long time, and not even one-tenth of the eggs hatched. Among the hatched animals there was at first a high mortality rate, especially in the second culture. Nevertheless, I obtained even now, when many larvae are still not metamorphosed, several hundred little frogs, all males except for a very few females, and few animals which can be sexed only on section, an investigation which has not yet been done. The need to study the problem even more exactly stimulated me to modify the procedures with Rana esculenta. The separation of males and females was undertaken first as the female had laid a portion of her eggs. The rest of the eggs were then fertilized, two days later. With the help of this procedure, it is possible to compare normal eggs with the eggs of the same animal which show one, two, or three days of postmaturity. By varying this procedure, it will be possible to achieve very reliable results. I cannot report in great detail on esculenta experiments, since the development had not proceeded far enough yet; only the one appears to me to have already progressed so that the postmature eggs are yielding larvae of significant size, which are almost exclusively males, while the normal culture contains almost equal quantities of males and females, observations which agree very well with my interpretation that in postmature eggs the regulating influence of fertilization is not as important. Furthermore, I would like to mention a defect in this addendum which could be attributed to my interpretation that parthenogenesis must have a sex-determining effect in a similar overall fashion. /214 Maupas, in his very interesting experiments on the propagation of rhabditids has arrived at the highly-interesting result that parthenogenetically propagating females produce protogynecic hermaphrodites, fertilized females, in contrast, pure females. It seems likely to me that fertilization operates here in an inhibitory fashion for development and thereby becomes a cause for the fact that development comes to a standstill with the formation of eggs.

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